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Characterization and Genome Study of Two Novel Bacteriophages Targeting Serratia Isolated from Ready-to-Eat Food

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Statement of the Problem: High microbial contamination of raw materials and increasing antibiotic resistance are prompting the food industry to search for new effective methods of minimally processed food (MPF) preservation. Production of MPF involves mild food processing methods. As a result, the level of microbial contamination in this type of product is higher compared to highly processed foods. Plant-based food products have a short shelf life, even at low storage temperatures, due to the presence of saprophytic bacteria. The search for food preservation methods has turned to the use of lytic bacteriophages. Studies show that the use of phages and their enzymes does not affect the sensory properties of food (i.e., taste, appearance, color, and odor). Due to their highly specific host preferences, the application of bacteriophages and their enzymes is a promising method for the detection and biocontrol of bacterial contamination in agriculture, medicine (phage therapy), and food production and processing.

Methodology & Theoretical Orientation: Bacteriophages targeting bacteria from the Serratia genus were isolated from municipal wastewater. In the double-layer plate test, visible plaques, that indicate the activity of bacteriophages were surrounded by a characteristic halo zone, which indicates the production of lytic enzymes. Bacteriophages' genomic DNA was isolated from lysates using the PureLinkTM RNA/DNA Mini Kit, followed by the next-generation sequencing analysis using nanopore technology (GridION long-read sequencer). The resulting consensus sequences were analyzed in bioinformatic software (i.a. Phanotate, Ublast, ViPTree, Prokka, RGI, PhageAI, VirSorter, Phigaro, and PhaGAA). Proteomic trees of the newly isolated phages were generated using the ViPTree server. In addition, the effects of selected environmental factors - active acidity (pH in the range from 3 to 12), and temperature (in the range from -20°C to 80°C) on the phages activity - were determined.

Results: According to the sequence analysis, the Serratia phage KKP 3708 genome consists of 40,461 bp linear dsDNA with a total G+C content of 52.9%. Among 55 predicted proteins, 23 are of unknown function, while the remaining were annotated as structural (portal, capsid, collar, and tail proteins), replication, and lysis proteins (holin). No genes associated with antibiotic resistance were found in the genome of the newly isolated phage. No tRNAs were found in the phage genome, suggesting that the Serratia phage KKP 3708 did not take over the host transcription/translation system, but uses host tRNAs for the synthesis of phage proteins. In addition, in the Serratia phage KKP 3708 genome neither the antibiotic resistance genes nor genes associated with integration into the host genome were located (markers of temperate bacteriophages). BLASTn analysis revealed that the Serratia phage KKP 3708 genome had high similarity with tailed Przondovirus phages from the Autographiviridae family.

Analysis of the Serratia phage KKP 3709 genome (67,890 bp linear dsDNA) showed no tRNAs. The overall G+C content of its genome is 49.8%. Among 194 predicted proteins, 130 are of unknown function, while the remaining were annotated as structural (portal, capsid, tail, collar, and baseplate proteins), replication, and lysis proteins (endolysin). No antibiotic resistance genes and other genes associated with integration into the host genome were located. BLASTn analysis revealed that the Serratia phage KKP 3709 genome had high similarity with tailed phages from the Myosmarvirus genus.

The bacteriophages exhibited high lytic activity in the temperature range from -20℃ to 40℃ and active acidity in the pH range from 3 to 12. In the case of Serratia phage KKP 3708, a significant reduction in phage titer was observed after incubation at 50℃ (a

reduction of 3.5 log orders) compared to the control cultured at 20℃. Temperatures above 70℃ resulted in the complete inactivation of bacteriophages. Serratia phage KKP 3709 maintained high stability in the range of -20℃ to 40℃. Incubation at 50℃ reduced the lytic activity by almost one and a half log orders. Exposure to 80℃ resulted in a decrease in the activity of almost six log orders compared to control conditions. For both phages, temperature affected the stability of bacteriophages more than active acidity. Incubation of Serratia phage KKP 3708 at the extremes of pH 3 and pH 12 resulted in a 1.5 log order reduction in phage titer compared to control conditions (pH 7). The Serratia phage KKP 3709 exhibited high stability over the range of active acidity. Incubation at extreme pH values reduced its activity by almost a log order (90%) compared to control conditions. Obtained results indicate that changes in the hydrogen ion concentration did not significantly affect the stability of the tested virions.

Conclusion: Genome analysis suggests that both phages do not encode genes associated with toxins or other virulence factors. Consequently, the newly isolated phages should be considered virulent bacteriophages. Virulent characteristics and no possible pathogen factors make them feasible to be potential candidates for food biopreservation. The high lytic activity of bacteriophages over a wide range of temperatures and pH allows their use in ready-to-eat food production.

Biography

Biotechnologist with a specialization in food biotechnology. Engineering and technical specialist in the Collection of Industrial Microbial Cultures - Microbiological Resources Center, which is part of the Department of Microbiology in the Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology - State Research Institute (IAFB). The use of bacteriophages in the food industry is her main area of interest. She is a Ph.D. Student conducting research on the synergistic effects of bacteriophages and high hydrostatic pressure technique for food preservation.

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